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(54) Title: MONOCLONAL ANTIBODIES AND VACCINES AGAINST EPITOPES ON THE EBOLA VIRUS GLYCOPROTEIN

(57) Abstract: In this application are described Ebola GP monoclonal antibodies and epitopes recognized by these monoclonal antibodies. Also provided are mixtures of antibodies of the present invention, as well as methods of using individual antibodies or mixtures thereof for the detection, prevention, and/or therapeutical threatment of Ebola virus infections in vitro and in vivo.

glycoprotein (sGP) that is synthesized in large amounts early during the course of infection (Volchkov et al., 1995, supra; Sanchez et al., 1996, supra; Sanchez et al., J. Infect. Dis. 179 (suppl. 1, S164, 1999). Following editing, the virion-associated transmembrane glycoprotein is proteolytically processed into 2 disulfide-linked products (Sanchez *et al.*, J. Virol. 72, 6442-6447, 1998). The amino-terminal product is referred to as GP_1 (140 kDa) and the carboxy-terminal cleavage product is referred to as GP_2 (26 kDa). GP_1 and membrane-bound GP, covalently associate to form a monomer of the GP spike found on the surfaces of virions (V. E. Volchkov et al., Proc. Natl. Acad. Sci. U.S.A. 95, 5762, 1998; A. Sanchez et al., J. Virol. 72, 6442, 1998). GP_1 is also released from infected cells in a soluble form (V. E. Volchkov. et al., Virology 245, 110, 1998). sGP and GP_1 are identical in their first 295 N-terminal amino acids, whereas the remaining 69 C-terminal amino acids of sGP and 206 amino acids of GP_1 are encoded by different reading frames. It has been suggested that secreted GP_1 or sGP may effectively bind antibodies that might otherwise be protective (Sanchez et al., 1996, supra; Volchkov et al. 1998, supra).

Ebola virus GP is a type I transmembrane glycoprotein. Comparisons of the predicted amino acid sequences for the GPs of the different Ebola virus strains show conservation of amino acids in the amino-terminal and carboxy-terminal regions with a highly variable region in the middle of the protein (Feldmann *et al.*, Virus Res. 24: 1-19, 1992). The GP of Ebola viruses are highly glycosylated and contain both N-linked and O-linked carbohydrates that contribute up to 50% of the molecular weight of the protein. Most

of the glycosylation sites are found in the central variable region of GP.

Other studies have also demonstrated limited efficacy of passively transferred polyclonal antibodies in protection against Ebola challenge (Mikhailov *et al*, 1994, Voprosi Virusologii, 39, 82-84; Jahrling *et al.*, 1996, Arch Virol, 11S, 135-140; Jahrling *et al.*, 1999, J Infect Dis, 179 (Suppl 1), S224-234; Kudoyarova-Zubavichene *et al.*, 1999, J Infect Dis, 179(Suppl 1), S218-223). However, it is difficult to determine the effective therapeutic dose of antibodies in different preparations of polyclonal antibodies. In addition, it is not known if monoclonal antibodies (MAbs) recognizing single epitopes on the Ebola GP are able to effectively neutralize or protect against Ebola virus *in vivo*.

SUMMARY OF THE INVENTION

This application describes protective GP-specific MAbs. The antibodies are classified into five groups based on competitive binding assays. Individual MAbs in these five groups were protective against Ebola challenge when administered prophylactically or therapeutically. Three of the epitopes bound by protective MAbs are linear sequences on GP_1 whereas the other two are conformational epitopes shared between GP_1 and sGP. Ten out of 14 MAbs identified in these five competition groups protected BALB/c mice from a lethal challenge with mouse-adapted Ebola Zaire virus when 100 ug of purified MAb was administered 24 hours before challenge (please see Table 3 in Examples below). Similar results were observed in a second mouse strain (C57BL/6). Protection from Ebola challenge decreased when the MAb dose was lowered to

50 or 25 ug (Please see Table 3 and Table 5 in Examples below). For the most effective MAbs, the amount required for protection was within an achievable human therapeutic dose of 3-5 mg/kg.

Some of the MAbs were effective even when administered up to 2 days after challenge (please see Table 3 in Examples below), after significant viral replication had occurred (M. Bray et al., J. Infect. Dis. 178, 651, 1998). None of the MAbs were protective when 100 ug was administered 3 days after challenge, when there are high viral titers (Bray et al., 1998, supra) and possibly irreversible damage of cells and organs.

The ability of the MAbs to inhibit plaque formation by Ebola virus, a standard assay of virus neutralization, did not always predict their protective efficacy. None of the protective MAbs inhibited plaque formation in the absence of complement (please see Table 6 in the Examples below).

Therefore, it is an object of the present invention to provide monoclonal antibodies which protect against Ebola virus and bind to epitopes on the Ebola virus GP. Such antibodies are, for instance, produced by any one of the cell lines deposited under the Budapest Treaty at American Type Culture Collection, Manassas, Virginia on July 20, 1999, EGP 13F6-1-2, assigned accession no. PTA-373, EGP6D3-1-1 assigned accession no. PTA-374, EGP 13-C6-1-1 assigned accession no. PTA-375, EGP 6D8-1-2 assigned accession no. PTA-376 and EGP 12B5-1-1 deposited on July 29, 1999 and assigned accession no. PTA-436 (Table 1).

Table 1

Monoclonal	Hybridoma	ATCC accession no.
MAb 6D8	EGP 6D8-1-2	PTA-376
MAb 13F6	EGP 13F6-1-2	PTA-373
MAb 12B5	EGP 12B5-1-1	PTA-436
MAb 13C6	EGP 13-C6-1-1	PTA-375
MAb 6D3.	EGP6D3-1-1	PTA-374

It is another object of the invention to provide for antibodies that are functionally equivalent to the antibodies listed above. These functionally equivalent antibodies substantially share at least one major functional property with an antibody listed above and herein described comprising: binding specificity to Ebola GP, protection against Ebola challenge when administered prophylactically or therapeutically, competition for same binding site on Ebola GP. The antibodies can be of any class such as IgG, IgM, or IgA or any subclass such as IgG1, IgG2a, and other subclasses known in the art. Further, the antibodies can be produced by any method, such as phage display, or produced in any organism or cell line, including bacteria, insect, mammal or other type of cell or cell line which produces antibodies with desired characteristics, such as humanized antibodies. The antibodies can also be formed by combining an Fab portion and a Fc region from different species.

The monoclonal antibodies of the present invention described below recognize epitopes on Ebola GP (SEQ ID NO: 1 and 2 describe the DNA and amino acid sequence, respectively, of Ebola GP used as an immunogen). Three epitopes are within the sequence extending from 389 to 493 and defined as:

HNTPVYKLDISEATQVEQHHRRTDNDSTASDTPSATTAAGPPKAENTNTSKSTD
FLDPATTTSPQNHSETAGNNNTHHQDTGEESASSGKLGLITNTIAGVAGLI
(SEQ ID NO:3). More specifically, the cell line EGP 13F6-1-2 produces a monoclonal antibody 13F6 which recognizes and binds to an amino acid sequence of GP corresponding to a region extending from 401 to 417 (SEQ ID NO:4), recognizing an epitope within this region corresponding to Glu-Gln-His-His-Arg-Arg-Thr-Asp-Asn (SEQ ID NO:5). The cell line EGP 6D8-1-2 produces a monoclonal antibody 6D8 which recognizes and binds to an amino acid sequence of GP corresponding to a region extending from 389 to 405 (SEQ ID NO:6), recognizing an epitope within this region corresponding to Val-Tyr-Lys-Leu-Asp-Ile-Ser-Glu-Ala (SEQ ID NO:7). The cell line EGP 12B5-1-1 produces a monoclonal antibody 12B5 which recognizes and binds to an amino acid sequence of GP corresponding to a region extending from 477 to 493 (SEQ ID NO:8), recognizing an epitope within this region corresponding to Leu-Ile-Thr-Asn-Thr-Ile-Ala-Gly-Val (SEQ ID NO:9). The antibodies produced by cell lines EGP 13C6-1-1, 13C6, and EGP 6D3-1-1, 6D3, recognize conformational epitopes in GP sequence that may comprise discontinuous Ebola virus amino acids that are conserved between Zaire and Ivory Coast viruses and found in the 295 amino terminus of the protein (SEQ ID NO:10).

It is another object of the present invention to provide for mixtures of antibodies according to the present invention, as well as to methods of using individual antibodies, or mixtures thereof for the prevention and/or therapeutic treatment of Ebola infections in vitro and in vivo, and/or for improved detection of Ebola infections.

It is yet another object of the present invention to treat or prevent Ebola virus infection by administering a therapeutically or prophylactically effective amount of one antibody of the present invention or a mixture of antibodies of the present invention to a subject in need of such treatment.

It is another object of the present invention to provide passive vaccines for treating or preventing Ebola virus infections comprising a therapeutically or prophylactically effective amount of the antibodies of the present invention which protect against Ebola virus, in combination with a pharmaceutically acceptable carrier or excipient.

It is yet another object of the present invention to provide a method for diagnosis of Ebola virus infection by assaying for the presence of Ebola in a sample using the antibodies of the present invention.

It is still another object of the present invention to provide novel immunoprobes and test kits for detection of Ebola virus infection comprising antibodies according to the present invention. For immunoprobes, the antibodies are directly or indirectly attached to a suitable reporter molecule, e.g., and enzyme or a radionuclide. The test kit includes a container holding one or more antibodies according to the present invention and instructions for using the antibodies for the purpose of binding to Ebola virus to form an immunological complex and detecting the formation of the immunological complex such that presence or absence of the immunological complex correlates with presence or absence of Ebola virus.

It is another object of the present invention to provide anti-idiotypic antibodies raised against one

of the present monoclonal antibodies for use as a vaccine to elicit an active anti-GP response.

It is yet another object of the present invention to provide antigenic epitopes as a component of a Ebola virus vaccine. The epitopes described above comprising SEQ ID NO:3-10, or conservative changes thereof which are still recognized by the antibodies, are useful for actively immunizing a host to elicit production of protective antibodies against Ebola.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description and appended claims, and accompanying drawings where:

Figure 1 illustrates the replicon VRegEboGP used make replicon particles EboGP-VRP. The particles were used to vaccinate mice for production of antibodies to Ebola GP.

Figure 2A and 2B Immunoprecipitation of ^{35}S-labeled Ebola GPs from supernatants of Vero cells infected with (A) EboGP-VRPs or (B) Ebola Zaire 1995 virus, with either the MAb 13F6 (Lane 1) or the MAb 13C6 (Lane 2). Both preparations contained secreted GP_1 and sGP. Disulfide-linked GP_1 and GP_2 comprise the spikes on the virions that are also present in the Ebola-infected preparation (B). The immunoprecipitation of GPs with 13F6 was identical to that observed with MAbs 6D8 and 12B5. MAb 6D3 had reactivities identical to MAb 13C6. GP proteins were resolved under reducing conditions on an 11% SDS polyacrylamide gel.

DETAILED DESCRIPTION

In the description that follows, a number of terms used in recombinant DNA, virology and immunology are extensively utilized. In order to provide a clearer and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

"Ebola viruses", members of the family Filoviridae, are associated with outbreaks of highly lethal hemorrhagic fever in humans and nonhuman primates. Human pathogens include Ebola Zaire, Ebola Sudan, and Ebola Ivory Coast. Ebola Reston is a monkey pathogen and is not considered a human pathogen. The natural reservoir of the virus is unknown and there are currently no available vaccines or effective therapeutic treatments for filovirus infections. The genome of Ebola virus consists of a single strand of negative sense RNA that is approximately 19 kb in length. This RNA contains seven sequentially arranged genes that produce 8 mRNAs upon infection. Ebola virions, like virions of other filoviruses, contain seven proteins: a surface glycoprotein (GP), a nucleoprotein (NP), four virion structural proteins (VP40, VP35, VP30, and VP24), and an RNA-dependent RNA polymerase (L) (Feldmann *et al.*(1992) *Virus Res.* 24, 1-19; Sanchez *et al.*,(1993) *Virus Res.* 29, 215-240; reviewed in Peters *et al.* (1996) *In* Fields Virology, Third ed. pp. 1161-1176. Fields, B. N., Knipe, D. M., Howley, P.M., *et al.* eds. Lippincott-Raven Publishers, Philadelphia). The glycoprotein of Ebola virus is unusual in that it

is encoded in two open reading frames. Transcriptional editing is needed to express the transmembrane form that is incorporated into the virion (Sanchez *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93, 3602-3607; Volchkov *et al*, (1995) *Virology* 214, 421-430). The unedited form produces a nonstructural secreted glycoprotein (sGP) that is synthesized in large amounts early during the course of infection. Little is known about the biological functions of these proteins and it is not known which antigens significantly contribute to protection and should therefore be used to induce an immune response.

The term "antibody" is art-recognized terminology and is intended to include molecules or active fragments of molecules that bind to known antigens. Examples of active fragments of molecules that bind to known antigens include Fab and $F(ab')_2$ fragments. These active fragments can be derived from an antibody of the present invention by a number of techniques. For example, purified monoclonal antibodies can be cleaved with an enzyme, such as pepsin, and subjected to HPLC gel filtration. The appropriate fraction containing Fab fragments can then be collected and concentrated by membrane filtration and the like. For further description of general techniques for the isolation of active fragments of antibodies, see for example, Khaw, B. A. et al. *J. Nucl. Med.* 23:1011-1019 (1982). The term "antibody" also includes bispecific and chimeric antibodies.

The language "monoclonal antibody" is art-recognized terminology. The monoclonal antibodies of the present invention can be prepared using classical cloning and cell fusion techniques. The immunogen (antigen) of interest, Ebola GP protein, is typically

administered (e.g. intraperitoneal injection) to wild type or inbred mice (e.g. BALB/c) or transgenic mice which produce desired antibodies, rats, rabbits or other animal species which can produce native or human antibodies. The immunogen can be administered alone, or mixed with adjuvant, or expressed from a vector (VEE replicon vector, vaccinia), or as DNA, or as a fusion protein to induce an immune response. Fusion proteins comprise the peptide against which an immune response is desired coupled to carrier proteins, such as β-galactosidase, glutathione S-transferase, keyhole limpet hemocyanin (KLH), and bovine serum albumin, to name a few. In these cases, the peptides serve as haptens with the carrier proteins. After the animal is boosted, for example, two or more times, the spleen is removed and splenocytes are extracted and fused with myeloma cells using the well-known processes of Kohler and Milstein (*Nature* 256: 495-497 (1975)) and Harlow and Lane (Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, New York 1988)). The resulting hybrid cells are then cloned in the conventional manner, e.g. using limiting dilution, and the resulting clones, which produce the desired monoclonal antibodies, cultured.

Monoclonal antibodies raised against Ebola GP as described in the Examples are listed in Table 1 above.

The term "epitope" is art-recognized. It is generally understood by those of skill in the art to refer to the region of an antigen, such as Ebola virus GP, that interacts with an antibody. An epitope of a peptide or protein antigen can be formed by contiguous or noncontinguous amino acid sequences of the antigen. Ebola GP, like many large proteins, contains many epitopes. Examples of Ebola GP epitopes recognized by antibodies of the present invention include the

region extending from 389 to 493 and defined as: HNTPVYKLDISEATQVEQHHRRTDNDSTASDTPSATTAAGPPKAENTNTSKSTD FLDPATTTSPQNHSETAGNNNTHHQDTGEESASSGKLGLITNTIAGVAGLI (SEQ ID NO:3). Continuous epitopes were found within 1) the amino acid sequence of GP corresponding to a region extending from 401 to 417 (SEQ ID NO:4), for example corresponding to Glu-Gln-His-His-Arg-Arg-Thr-Asp-Asn (SEQ ID NO:5), 2) the amino acid sequence of GP corresponding to a region extending from 389 to 405 (SEQ ID NO:6), for example corresponding to Val-Tyr-Lys-Leu-Asp-Ile-Ser-Glu-Ala (SEQ ID NO:7), and 3) the amino acid sequence of GP corresponding to a region extending from 477 to 493 (SEQ ID NO:8), for example Leu-Ile-Thr-Asn-Thr-Ile-Ala-Gly-Val (SEQ ID NO:9). The epitopes or peptides recognized by the antibodies of the present invention and conservative substitutions of these peptides which are still recognized by the antibody are an embodiment of the present invention. These peptides offer a convenient method for eluting GP bound to MAb 6D8, 13F6, or 12B5 on immunoaffinity columns. For example, when an antibody which recognizes the epitope for MAb 6D8, 13F6 or 12B5 is used in an immunoaffinity column to purify Ebola GP, the peptide recognized by the antibody can be added to the immunoaffinity column to elute the Ebola GP. Further truncation of these epitopes may be possible since antigenic epitopes have been reported to be represented by as few as five amino acid residues.

Epitope mapping studies described in this application defined five competition groups of MAbs. Antibodies which compete with the monoclonal antibodies of the present invention for binding to GP are considered to recognize the epitopes of the antibodies and are considered equivalent to the

antibodies of the present invention. The MAbs 13C6 and 6D3 recognize conformational epitopes comprising discontinuous Ebola virus amino acids. Antibodies which compete with MAbs 13C6 and 6D3 for binding to Ebola GP are considered to recognize discontinuous epitopes and are considered equivalent to the antibodies of the present invention. Assays for determining whether or not an antibody competes with an antibody of the present invention are known to a person with ordinary skill in the art and are described below. Table 2 below defines functional criteria of each of the monoclonal antibodies identified in the Examples below.

Table 2. Epitopes Bound by Ebola GP MAbs.

Competition Group	Ebola Viruses with Epitope*	Ebola GPs with Epitope†	Epitope Sequence on Ebola GP‡	Amino Acids§
1	Z	GP_1	ATQV**EQHHRRTDN**DSTA	401-417
2	Z	GP_1	HNTP**VYKLDISEA**TQVE	389-405
3	Z	GP_1	GKLG**LITNTIAGV**AGLI	477-493
4	Z, IC, S	GP_1, sGP	discontinuous	1-295
5	Z, IC	GP_1, sGP	discontinuous	1-295

*Reactivities of MAbs with Ebola Zaire (Z, isolates from 1976 and 1995), Sudan (S), and Ivory Coast (IC) viruses in ELISA.

†Determined by western blot reactivity with Ebola Zaire 1995 virions or by immunoprecipitation (Fig. 2).

‡MAbs bound two consecutive peptide sequences immobilized on SPOTS membranes. Each peptide was 13 amino acids long and had a 9 amino acid overlap with the preceding and subsequent peptides. Sequences in bold indicate the 9 amino acid overlapping consensus sequence found on both peptides bound by the MAbs. Peptides containing the entire amino acid sequence shown also competed the binding of MAbs to Ebola virions in ELISA.

[5]Amino acid numbers based on the GP sequence from Genbank (accession number U23187, A. Sanchez, S. G. et al. (1996) *Proc. Natl. Acad. Sci., USA* 93, 3602).

By further mapping of the binding site of the monoclonal antibodies described in this application other peptides useful as a vaccine or a therapeutic can be predicted. Therefore, in another aspect, this invention relates to a method for identifying protective antigenic epitopes the method comprising (i) reacting a monoclonal antibody described in this application to different overlapping fragments encompassing the complete antigen, (ii) identifying a fragment to which the protective antibody binds, (iii) narrowing the region containing sites further by reacting the monoclonal with smaller overlapping fragments encompassing the region identified in (ii), and (iv) choosing peptides to which the antibody binds as possible antigenic epitopes. The peptides can then be assayed for their ability to protect an animal from disease, or to reduce the severity of disease. Peptides defining antigenic protective epitopes can be used in a vaccine as described below and in the Examples.

The epitopes or peptides on Ebola GP to which the monoclonal antibodies bind can constitute all or part of an eventual active vaccine candidate. An active vaccine or therapeutic candidate might comprise these peptide sequences and others. These might be delivered as synthetic peptides, or as fusion proteins, alone or co-administered with cytokines and/or adjuvants or carriers safe for human use, e.g. aluminum hydroxide, to increase immunogenicity. In addition, sequences such as ubiquitin can be added to increase antigen processing for more effective immune responses.

The present invention also pertains to hybridomas producing antibodies which bind to an epitope of Ebola GP. The term "hybridoma" is art recognized and is understood by those of ordinary skill in the art to refer to a cell produced by the fusion of an antibody-producing cell and an immortal cell, e.g. a multiple myeloma cell. This hybrid cell is capable of producing a continuous supply of antibody. See the definition of "monoclonal antibody" above and the Examples below for a more detailed description of the method of fusion.

The present invention still further pertains to a method for detecting Ebola GP in a sample suspected of containing Ebola GP. The method includes contacting the sample with an antibody which binds an epitope of Ebola GP, allowing the antibody to bind to Ebola GP to form an immunological complex, detecting the formation of the immunological complex and correlating the presence or absence of the immunological complex with the presence or absence of Ebola GP in the sample. The sample can be biological, environmental or a food sample.

The language "detecting the formation of the immunological complex" is intended to include discovery of the presence or absence of Ebola GP in a sample. The presence or absence of Ebola GP can be detected using an immunoassay. A number of immunoassays used to detect and/or quantitate antigens are well known to those of ordinary skill in the art. See Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, New York 1988 555-612). Such immunoassays include antibody capture assays, antigen capture assays, and two-antibody sandwich assays. These assays are commonly used by those of ordinary skill in the art. In an antibody capture assay, the antigen is attached to solid

support, and labeled antibody is allowed to bind. After washing, the assay is quantitated by measuring the amount of antibody retained on the solid support. A variation of this assay is a competitive ELISA wherein the antigen is bound to the solid support and two solutions containing antibodies which bind the antigen, for example, serum from an Ebola virus vaccinee and a monoclonal antibody of the present invention, are allowed to compete for binding of the antigen. The amount of monoclonal bound is then measured, and a determination is made as to whether the serum contains anti Ebola GP antibodies. This competitive ELISA can be used to indicate immunity to known protective epitopes in a vaccinee following vaccination.

In an antigen capture assay, the antibody is attached to a solid support, and labeled antigen is allowed to bind. The unbound proteins are removed by washing, and the assay is quantitated by measuring the amount of antigen that is bound. In a two-antibody sandwich assay, one antibody is bound to a solid support, and the antigen is allowed to bind to this first antibody. The assay is quantitated by measuring the amount of a labeled second antibody that can bind to the antigen.

These immunoassays typically rely on labeled antigens, antibodies, or secondary reagents for detection. These proteins can be labeled with radioactive compounds, enzymes, biotin, or fluorochromes. Of these, radioactive labeling can be used for almost all types of assays and with most variations. Enzyme-conjugated labels are particularly useful when radioactivity must be avoided or when quick results are needed. Biotin-coupled reagents usually are detected with labeled streptavidin.

Streptavidin binds tightly and quickly to biotin and can be labeled with radioisotopes or enzymes. Fluorochromes, although requiring expensive equipment for their use, provide a very sensitive method of detection. Antibodies useful in these assays include monoclonal antibodies, polyclonal antibodies, and affinity purified polyclonal antibodies. Those of ordinary skill in the art will know of other suitable labels which may be employed in accordance with the present invention. The binding of these labels to antibodies or fragments thereof can be accomplished using standard techniques commonly known to those of ordinary skill in the art. Typical techniques are described by Kennedy, J. H., *et al.*,1976 (*Clin. Chim. Acta* 70:1-31), and Schurs, A. H. W. M., *et al.* 1977 (*Clin. Chim Acta* 81:1-40). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, and others, all of which are incorporated by reference herein.

The language "biological sample" is intended to include biological material, e.g. cells, tissues, or biological fluid. By "environmental sample" is meant a sample such as soil and water. Food samples include canned goods, meats, and others.

Yet another aspect of the present invention is a kit for detecting Ebola virus in a biological sample. The kit includes a container holding one or more antibodies which binds an epitope of Ebola GP and instructions for using the antibody for the purpose of binding to Ebola GP to form an immunological complex and detecting the formation of the immunological complex such that the presence or absence of the immunological complex correlates with presence or absence of Ebola virus in the sample. Examples of

containers include multiwell plates which allow simultaneous detection of Ebola virus in multiple samples.

As described in greater detail in the examples, the present inventors have isolated five monoclonal antibodies which bind to five epitopes on Ebola GP and display in vitro and/or in vivo Ebola virus protective properties. Significantly, the reactivity of the MAbs is applicable against a broad variety of different wild type and laboratory Ebola strains of different types. Wild type strains include for example Ebola Ivory Coast, Ebola Zaire 1976 (Mayinga isolate), Ebola Zaire 1975, and Ebola Sudan (Boniface). Laboratory strains can be derived from wild type strains and include those which have been passaged or animal adapted strains such as mouse-adapted Ebola.

Given these results, monoclonal antibodies according to the present invention are suitable both as therapeutic and prophylactic agents for treating or preventing Ebola infection in susceptible Ebola-infected subjects. Subjects include rodents such as mice or guinea pigs, monkeys, and other mammals, including humans.

In general, this will comprise administering a therapeutically or prophylactically effective amount of one or more monoclonal antibodies of the present invention to a susceptible subject or one exhibiting Ebola infection. Any active form of the antibody can be administered, including Fab and $F(ab')_2$ fragments. Antibodies of the present invention can be produced in any system, including insect cells, baculovirus expression systems, chickens, rabbits, goats, cows, or plants such as tomato, potato, banana or strawberry. Methods for the production of antibodies in these systems are known to a person with ordinary skill in

the art. Preferably, the antibodies used are compatible with the recipient species such that the immune response to the MAbs does not result in clearance of the MAbs before virus can be controlled, and the induced immune response to the MAbs in the subject does not induce "serum sickness" in the subject. Preferably, the MAbs administered exhibit some secondary functions such as binding to Fc receptors of the subject.

Treatment of individuals having Ebola infection may comprise the administration of a therapeutically effective amount of Ebola antibodies of the present invention. The antibodies can be provided in a kit as described below. The antibodies can be used or administered as a mixture, for example in equal amounts, or individually, provided in sequence, or administered all at once. In providing a patient with antibodies, or fragments thereof, capable of binding to Ebola GP, or an antibody capable of protecting against Ebola virus in a recipient patient, the dosage of administered agent will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition, previous medical history, etc.

In general, it is desirable to provide the recipient with a dosage of antibody which is in the range of from about 1 pg/kg-100 pg/kg, 100 pg/kg-500 pg/kg, 500 pg/kg-1 ng/kg, 1 ng/kg-100 ng/kg, 100 ng/kg-500 ng/kg, 500 ng/kg- 1 ug/kg, 1 ug/kg- 100 ug/kg, 100 ug/kg-500 ug/kg, 500 ug/kg- 1 mg/kg, 1 mg/kg-50 mg/kg, 50 mg/kg-100 mg/kg, 100 mg/kg-500 mg/kg, 500 mg/kg-1 g/kg, 1 g/kg-5 g/kg, 5 g/kg-10 g/kg (body weight of recipient), although a lower or higher dosage may be administered.

In a similar approach, another therapeutic use of the monoclonal antibodies of the present invention is the active immunization of a patient using an anti-idiotypic antibody raised against one of the present monoclonal antibodies. Immunization with an anti-idiotype which mimics the structure of the epitope could elicit an active anti-GP response (Linthicum, D.S. and Farid, N.R., Anti-Idiotypes, Receptors, and Molecular Mimicry (1988), pp 1-5 and 285-300).

Likewise, active immunization can be induced by administering one or more antigenic and/or immunogenic epitopes as a component of a subunit vaccine. Vaccination could be performed orally or parenterally in amounts sufficient to enable the recipient to generate protective antibodies against this biologically functional region, prophylactically or therapeutically. The host can be actively immunized with the antigenic/immunogenic peptide in pure form, a fragment of the peptide, or a modified form of the peptide. One or more amino acids, not corresponding to the original protein sequence can be added to the amino or carboxyl terminus of the original peptide, or truncated form of peptide. Such extra amino acids are useful for coupling the peptide to another peptide, to a large carrier protein, or to a support. Amino acids that are useful for these purposes include: tyrosine, lysine, glutamic acid, aspartic acid, cyteine and derivatives thereof. Alternative protein modification techniques may be used e.g., NH_2-acetylation or COOH-terminal amidation, to provide additional means for coupling or fusing the peptide to another protein or peptide molecule or to a support.

The antibodies capable of protecting against Ebola virus are intended to be provided to recipient subjects in an amount sufficient to effect a reduction

in the Ebola virus infection symptoms. An amount is said to be sufficient to "effect" the reduction of infection symptoms if the dosage, route of administration, etc. of the agent are sufficient to influence such a response. Responses to antibody administration can be measured by analysis of subject's vital signs.

A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient patient. Such an agent is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient.

The compounds of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby these materials, or their functional derivatives, are combined in admixture with a phamaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in *Remington's Pharmaceutical Sciences* (16th ed., Osol, A. ed., Mack Easton Pa. (1980)). In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the above-described compounds together with a suitable amount of carrier vehicle.

Additional pharmaceutical methods may be employed to control the duration of action. Control release preparations may be achieved through the use of polymers to complex or absorb the compounds. The controlled delivery may be exercised by selecting

appropriate macromolecules (for example polyesters, polyamino acids, polyvinyl, pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, or protamine sulfate) and the concentration of macromolecules as well as the method of incorporation in order to control release. Another possible method to control the duration of action by controlled release preparations is to incorporate the compounds of the present invention into particles of a polymeric material such as polyesters, polyamino acids, hydrogels, poly(lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating these agents into polymeric particles, it is possible to entrap these materials in microcapsules prepared, for example, interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly(methylmethacylate)-microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* (1980).

Administration of the antibodies disclosed herein may be carried out by any suitable means, including parenteral injection (such as intraperitoneal, subcutaneous, or intramuscular injection), *in ovo* injection of birds, orally, or by topical application of the antibodies (typically carried in a pharmaceutical formulation) to an airway surface. Topical application of the antibodies to an airway surface can be carried out by intranasal administration (e.g., by use of dropper, swab, or inhaler which deposits a pharmaceutical formulation intranasally). Topical

application of the antibodies to an airway surface can also be carried out by inhalation administration, such as by creating respirable particles of a pharmaceutical formulation (including both solid particles and liquid particles) containing the antibodies as an aerosol suspension, and then causing the subject to inhale the respirable particles. Methods and apparatus for administering respirable particles of pharmaceutical formulations are well known, and any conventional technique can be employed. Oral administration may be in the form of an ingestable liquid or solid formulation.

The treatment may be given in a single dose schedule, or preferably a multiple dose schedule in which a primary course of treatment may be with 1-10 separate doses, followed by other doses given at subsequent time intervals required to maintain and or reinforce the response, for example, at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. Examples of suitable treatment schedules include: (i) 0, 1 month and 6 months, (ii) 0, 7 days and 1 month, (iii) 0 and 1 month, (iv) 0 and 6 months, or other schedules sufficient to elicit the desired responses expected to reduce disease symptoms, or reduce severity of disease.

The present invention also provides kits which are useful for carrying out the present invention. The present kits comprise a first container means containing the above-described antibodies. The kit also comprises other container means containing solutions necessary or convenient for carrying out the invention. The container means can be made of glass, plastic or foil and can be a vial, bottle, pouch,

tube, bag, etc. The kit may also contain written information, such as procedures for carrying out the present invention or analytical information, such as the amount of reagent contained in the first container means. The container means may be in another container means, e.g. a box or a bag, along with the written information.

The contents of all cited references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference.

Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

The following MATERIALS AND METHODS were used in the examples that follow.

Cell lines and Viruses

BHK (ATCC CCL 10), Vero 76 (ATCC CRL 1587), and Vero E6 (ATCC CRL 1586) cell lines were maintained in minimal essential medium with Earle's salts, 10% heat-inactivated fetal bovine serum, and 50 ug/ml gentamicin sulfate. Mouse hybridoma cell lines were maintained in Optimem medium (Life Technologies, Rockville, Maryland).

A stock of the Zaire strain of Ebola virus originally isolated from a patient in the 1976 Ebola outbreak (isolate Mayinga) and passaged intracerebrally 3 times in suckling mice and 2 times in Vero cells was adapted to adult mice through serial passage in progressively older suckling mice (Bray *et al.*, J. Infect. Dis. 178, 651-661, 1998). A plaque-purified ninth-mouse-passage isolate which was

uniformly lethal for adult mice ("mouse-adapted virus") was propagated in Vero E6 cells, aliquotted, and used in all mouse challenge experiments.

Ebola virus antigens used for characterization of monoclonal antibodies were prepared from the following virus seed stocks that were kindly provided by Dr. Peter Jahrling at USAMRIID: the Zaire 1995 strain of Ebola virus isolated from a patient in the 1995 outbreak and passaged 2 times in Vero E6 cells and 2 times in Vero cells; the Zaire 1976 strain of Ebola virus isolated from a patient in the 1976 Ebola outbreak (isolate Mayinga); the Sudan strain of Ebola virus (isolate Boniface) passaged 1 time in a guinea pig, and 3 times in Vero cells; the Ivory Coast strain of Ebola virus obtained from the Center for Disease Control (CDC #807212) and passaged 4 times in Vero E6 cells and 1 time in Vero cells.

Production of monoclonal antibodies

Five BALB/c mice were injected subcutaneously at the base of the neck with 2 x 10^6 focus-forming units of Venezuelan equine encephalitis (VEE) virus replicons encoding the glycoprotein (EboGP-VRP) from the Mayinga isolate of the Zaire strain of Ebola virus. EboGP-VRP particles were packaged and purified as described (Pushko *et al.*, 1997 In Vacines 97, pp.253-258. Cold Spring Harbor, N.Y.). Mice received 2 additional subcutaneous injections at one month intervals. ELISA titers to Ebola virus were measured after the third injection and the best two responders received an intravenous injection of 1 x 10^7 focus-forming units of Ebola GP replicons (EboGP-VRP) in the tail vein 21 days after the third subcutaneous injection. Three days after the final immunization, spleens were removed and used for fusion to P3X63Ag8:653 myeloma

cells as previously described (Stiles *et al.*, Toxicon 29, 1195-1204, 1991). Hybridoma culture supernatants were screened for the presence of antibodies to the Ebola GP by ELISA and by indirect immunofluorescence with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse antibodies as described below. Positive hybridoma cultures were cloned twice by limiting dilution. Large-scale preparations of MAbs were obtained by culturing hybridoma cell lines in serum-free medium in T150 Integra Celline flasks and purifying the MAbs from the supernatants over Protein G affinity columns (Pharmacia, Piscataway, NJ). Purified antibodies were dialyzed in PBS and quantitated using a BCA protein assay kit (Pierce, Rockford, IL).

Characterization of MAbs

ELISA

Enzyme-linked immunosorbent assays (ELISA) were performed essentially as described (Hevey *et al.*, Virology 239: 206-216, 1997). For screening of MAbs, 96-well PVC plates were coated overnight at 4°C with 0.05 ml of irradiated, sucrose-purified Ebola Zaire 1995 virions (10-20 ug/ml in PBS). For determining the cross-reactivities of the MAbs with other filovirus isolates, PVC plates were coated with either irradiated, sucrose-purified Ebola Zaire 1976 (Mayinga isolate), Ebola Zaire 1995, Ebola Ivory Coast, Ebola Sudan (Boniface). Plates were washed once with PBS containing 0.02% Tween-20 (PBST) and nonspecific binding was blocked by adding 0.25 ml of PBST containing 5% nonfat dry milk (PBSTM) to each well and incubating at room temperature for 1-2 hours. After washing the plates five times with 0.2 ml of PBST, 0.05 ml of undiluted hybridoma culture supernatants or

purified MAbs in PBSTM were added to wells containing antigen and plates were incubated for 2 h at room temperature. Bound MAbs were detected using horseradish peroxidase conjugated goat anti-mouse IgA + IgG + IgM (H+L) secondary antibodies and 2, 2'-Azinobis-[3-ethylbenzothizoline-6-sulfonic acid] diammonium salt (ABTS) substrate (Kirkegaard and Perry Laboratories, KPL, Gaithersburg, MD).

Indirect immunofluroscence antibody (IFA) assays

To determine whether the hybridoma cells produced MAbs that recognized either GP or sGP of Ebola virus, Mabs were reacted with Ebola GP-infected BHK cells. BHK cells were infected with EboGP-VRP, or with an irrelevant control replicon expressing the Lassa nucleoprotein, at a multiplicity of infection of 1 to 3 infectious units/cell. Cells were harvested with trypsin 17 h post-infection, washed 2 times in PBS, and diluted to 2 x 10^5 cells/ml in PBS. Thirty microliters of the cell suspension was applied to glass spot-slides and the slides were allowed to air dry. Cells were fixed with acetone at -20°C for 15 min and air-dried. Slides were stored at -70°C until needed. Twenty microliters of undiluted hybridoma culture supernatants were added, and the slides were incubated for 30 min at room temperature. Excess antibodies were removed from the cells by washing the slides in PBS for 30 min. Twenty microliters of fluorescein-labeled goat anti-mouse IgA + IgG + IgM (H+L) antibodies (50 ug/ml; KPL) was added to the cells and the slides were incubated for 30 min at room temperature. Excess secondary antibodies were removed from the cells by washing the slides for 30 min in PBS. The PBS was removed from the cells, and one drop of mounting medium (KPL) was added to each of the cell spots. Coverslips were added to the slides and the

staining patterns were viewed using a fluorescent microscope.

Metabolic labeling of Ebola virus proteins and radioimmunoprecipitation of Ebola GP proteins

Vero E6 cells (75 cm^2 flasks) were infected with the Zaire 1995 strain of Ebola virus at a multiplicity of infection of 1 to 3 plaque-forming units/cell. After 24 hours of infection, the growth medium was removed and cells were starved for 30 minutes in medium lacking methionine and cysteine. To label viral proteins, cells were incubated for 24 hours in MEM medium containing 2% heat-inactivated FBS, 0.1 mCi/ml ^{35}S-labeled methionine and 0.1 mCi/ml ^{35}S-labeled cysteine. The cell medium was harvested and centrifuged to remove cell debris (15 min at 1500 x g). Labeled Ebola virions were obtained by pelleting the clarified supernatant over a 20% sucrose cushion (3 h at 36,000 rpm in a SW41 rotor) and suspending the pelleted virions in Zwittergent lysis buffer. Ebola-infected cell lysates were obtained 24 hours after labeling by lysing infected cell monolayers in Zwittergent Lysis buffer.

To immunoprecipitate Ebola GP from EboGP-VRP-infected cells, Vero cells (75 cm^2 flasks) were infected with EboGP-VRP at a multiplicity of infection of 1 to 3 infectious units/cell. After 16 h of infection, cells were starved for 30 minutes in medium lacking methionine and cysteine. To label proteins, cells were incubated for 4 hours in MEM medium containing 2% heat-inactivated FBS, 0.1 mCi/ml ^{35}S-labeled methionine and 0.1 mCi/ml ^{35}S-labeled cysteine. Ebola GP mouse MAbs were used to immunoprecipitate Ebola GP proteins from the labeled cell lysates or supernatants.

Western Blot analysis

Unlabeled Ebola Zaire 1995 virion proteins were resolved on a 10% SDS-polyacrylamide gel and the proteins were transferred to Immobilon-P PVDF membranes. Nonspecific binding sites were blocked by incubating the membranes overnight at 4°C in PBSTM. Purified MAb (10 ug/ml in PBSTM) were added to the membranes for 1 hour at room temperature. The membranes were then incubated with horseradish peroxidase-conjugated goat anti-mouse IgA + IgG + IgM (H+L) secondary antibodies (1 ug/ml in PBSTM) for 1 hour at room temperature, and the ECL Western blot chemiluminescence kit (Amersham) was used to detect bound MAbs.

Isotype determination

Antibody subclasses were determined by ELISA. Briefly, 96-well plates were coated with anti-IgG, IgA or IgM heavy-chain specific antibodies (100 ng/well, KPL) and incubated with hybridoma culture supernatants. The subtype of the MAb was detected by using anti-IgG1 (Zymed, South San Francisco, CA), IgG2a, IgG2b, IgG3 (Cappel, Durham, NC), IgM (KPL), or IgA (Sigma, St. Louis, MO) heavy-chain specific antibodies conjugated to alkaline phosphatase.

Biotinylation of MAbs and competitive binding assays

MAbs were biotinylated using an EZ-Link™ Sulfo-NHS-LC-Biotinylation kit (Pierce, Rockford, IL) according to the manufacturer's instructions. Competitive binding between unlabeled and biotin-labeled MAbs was performed by reacting a 20-fold excess of unlabeled MAb and 1 to 200 micrograms of biotin-labeled MAb with sucrose-purified, irradiated Ebola Zaire 1995 virus bound to PVC plates. The results of the competition experiment were evaluated

by ELISA. The concentrations of biotinylated MAbs used in the competition assays were previously determined to be in the linear portion of their binding curve to Ebola virus antigen.

In vitro plaque-reduction neutralization assay

Plaque assays were performed using confluent Vero-E6 cells. To evaluate the presence of Ebola-neutralizing antibodies, four-fold serial dilutions of MAbs were mixed with 100 pfu of mouse-adapted Ebola virus at 37°C for 1 h, and used to infect Vero E6 cells. Cells were covered with an agarose overlay (Moe, J. *et al.* (1981) J. Clin. Microbiol. 13:791-793) and a second overlay containing 5% neutral red solution in PBS or agarose was added 6 days later. Plaques were counted the following day. Endpoint titers were determined to be the last dilution of MAb that reduced the number of plaques by 80% of the control wells.

SPOTS Peptide analysis.

To identify the protein sequences recognized by the MAbs, 166 peptides from the Ebola virus Zaire glycoprotein sequence were synthesized on membranes. Each peptide was 13 amino acids long and had a 9 amino acid overlap with the preceding and subsequent peptides. Peptides were synthesized directly on SPOTS membranes by Genosys, Inc. Two identical membranes were synthesized. Membranes were washed with methanol and then with PBS-0.02% Tween 20 (PBST), blocked overnight at 4°C in PBST + 5% nonfat dry milk (PBSTM), and rinsed in PBST. Ebola virus-specific MAbs were diluted to 5 micrograms/ml in PBSTM milk. Membranes were incubated with 25 ml of MAb for 1.5 h at room temperature, washed for 15 minutes in PBST and then twice more for 5 minutes each. The secondary antibody

(affinity purified, peroxidase-labeled goat anti-mouse IgA+IgM+IgG (H+L), Kirkegaard-Perry Labs, Inc. Catalog Number 074-1807, Lot SM055) was diluted 1:1000 in PBSTM and reacted with the membrane for 1.5 hrs at room temperature. The membrane was washed three times in PBST as described above. Lumi GLO chemiluminescent substrate (Kirkegaard Perry Catalog No. 54-61-00, Lot WD033) was prepared according to manufacturer's instructions and added to the membrane for 1 minute at room temperature. The membrane was exposed to Polaroid film using Amersham's ECL camera for various times. Positive spots were white against a black background. Membranes were reused after stripping. Stripping the membrane was performed by rinsing three times for 10 minutes each in 20 ml of MilliQ water, followed by dimethylformamide, and then twice in 20 ml MilliQ water. Three 10 minute washes in 20 ml regeneration buffer A (8M urea, 1% SDS, 0.1% 2-mercaptoethanol) were followed by three 10 minute washes in regeneration buffer B (50% ethanol, 10% acetic acid) and then by two 10 minute washes each in 20 ml methanol followed by PBST. Membranes were then blocked as described above.

Evaluation of MAbs in Mice

Administration of MAbs

To determine the prophylactic benefit of Ebola GP MAbs, purified MAbs or combinations of MAbs were injected intraperitoneally into BALB/c or C57BL/6 mice 24 h prior to challenge with mouse-adapted Ebola Zaire virus. For examination of therapeutic effects of MAbs, 100 micrograms of purified antibody, or various concentrations of combined MAbs, were injected intraperitoneally into BALB/c and C57BL/6 mice either 1, 2, or 3 days after challenge with mouse-adapted

Ebola Zaire virus. All antibodies were diluted in sterile PBS and 0.2 ml was injected into each mouse.

Ebola Infection of Mice

Mice were transferred to a BSL-4 containment area and challenged by intraperitoneal inoculation of 10 pfu of mouse-adapted Ebola Zaire 1976 virus (approximately 300 times the dose lethal for 50% of adult mice). Virus was diluted in EMEM medium without FBS. Animals were monitored for morbidity and mortality for 28 days post-infection.

Production of EboGP-VRP

The GP gene of Ebola Zaire was previously sequenced by Sanchez *et al.* (1993, *supra*) and has been deposited in GenBank (accession number L11365). A plasmid encoding the VEE replicon vector containing a unique ClaI site downstream from the 26S promoter was described previously (Davis, N. L. *et al.*, (1996) *J. Virol.* 70, 3781-3787; Pushko, P. *et al.* (1997) *Virology* 239, 389-401). The Ebola GP gene from the Ebola Zaire 1976 virus were derived from PS64-based plasmid (Sanchez, A. *et al.* (1989) *Virology* 170, 81-91; Sanchez, A. *et al.* (1993) *Virus Res.* 29, 215-240). From this plasmid, the BamHI-KpnI (2.4 kb) fragment containing the GP gene was subcloned into a shuttle vector that had been digested with BamHI and EcoRI (Davis *et al.* (1996) *supra;* Grieder, F. B. *et al.* (1995) *Virology* 206, 994-1006). For cloning of the GP gene, overhanging ends produced by KpnI (in the GP fragment) and EcoRI (in the shuttle vector) were made blunt by incubation with T4 DNA polymerase according to methods known in the art. From the shuttle vector, the GP gene was subcloned as ClaI-fragments into the ClaI site of the replicon clone, resulting in a plasmid encoding the GP gene in place of the VEE

structural protein genes downstream from the VEE 26S promoter, resulting in the replicon construct, VRepEboGP.

The Ebola virus GP gene cloned into the VEE replicon was sequenced. Changes in the DNA sequence relative to the sequence published by Sanchez *et al.* (1993) are described relative to the nucleotide (nt) sequence number from GenBank (accession number L11365).

The nucleotide sequence we obtained for Ebola virus GP (SEQ ID NO:1) differed from the GenBank sequence by a transition from A to G at nt 8023. This resulted in a change in the amino acid sequence from Ile to Val at position 662 (SEQ ID NO: 2).

Transfection of the replicon construct, VRepEboGP along with helper RNAs containing sequences necessary for packaging of the viral replicon transcripts will result in the production of virus-like particles containing replicon RNAs, such as EboGP-VRP. These packaged replicons will infect host cells and initiate a single round of replication resulting in the expression of the Ebola proteins in said infected cells.

Example 1

Production and characterization of Ebola GP MAbs

To obtain MAbs specific for the glycoprotein of Ebola virus, mice were vaccinated with VEE virus replicon particles (VRP) that express Ebola GP (EboGP-VRP). Spleen cells from two mice that were vaccinated with with EboGP-VRP were pooled and fused with P3X63Ag8:653 myeloma cells as described in Methods. In order to detect hybridomas producing antibodies that reacted with the GP of Ebola virus, 1,738 hybridoma supernatants were screened by ELISA for their ability to react with Ebola Zaire virion

Ebola GP (Example 3, Table 4). Fourteen of the MAbs tested in competition assays reacted with 5 different epitopes (Table 5). Ten of these 14 MAbs protected both BALB/c and C57BL/6 mice from a lethal challenge with mouse-adapted Ebola Zaire virus when 100 ug of purified MAb was administered 24 hours before challenge (Tables 3 and 5), demonstrating that antibodies that bind to any one of these Ebola GP epitopes can protect against lethal challenge.

To determine the effective dose of the protective MAbs, BALB/c mice were injected with either 50 ug, 25 ug, or 12.5 ug of purified Ebola GP MAbs 24 h prior to challenge with a lethal dose of mouse-adapted Ebola Zaire virus. For all of the MAbs examined, protection from Ebola challenge decreased when the MAb dose was lowered to 50 or 25 ug (Table 3). No protection was observed for any of the mice that received 12.5 ug of MAb (data not shown). For the most effective MAbs, the amount required for protection was within an achievable human therapeutic dose of 3-5 mg/kg.

To determine if the MAbs could be used therapeutically to treat mice that had already been infected with the Ebola virus, 100 ug of purified MAbs were injected into BALB/c or C57BL/6 mice either 1, 2, or 3 days after a lethal challenge with mouse-adapted Ebola Zaire virus. All of the MAbs that demonstrated protective efficacy when administered 1 day prior to challenge were also effective therapeutically when administered 1 day after challenge (Table 3). Some of the MAbs were effective even when administered up to 2 days after challenge (Table 3), after significant viral replication had occurred (M. Bray et al., J.

Table 3. Protective Efficacy of Ebola GP Monoclonal Antibodies.

Competition Group	MAb Designation	Day MAb Administered[1]	BALB/c S/T[2] (100 ug)	BALB/c S/T[2] (50 ug)	BALB/c S/T[2] (25 ug)	C57BL/6 S/T[2] (100 ug)
1	13F6 (IgG2a)	-1	10/10	7/10	6/10	9/10
		+1	10/10	---	---	9/10
		+2	3/10	---	---	2/10
2	6D8 (IgG2a)	-1	10/10	6/10	3/10	9/10
		+1	10/10	---	---	9/10
		+2	6/10	---	---	5/10
3	12B5 (IgG1)	-1	6/10	2/10	0/10	6/10
		+1	8/10	---	---	6/10
		+2	1/10	---	---	1/10
4	13C6 (IgG2a)	-1	10/10	7/10	3/10	9/10
		+1	10/10	---	---	10/10
		+2	8/10	---	---	9/10
5	6D3 (IgG2a)	-1	9/10	6/10	2/10	8/10
		+1	10/10	---	---	9/10
		+2	9/10	---	---	8/10
---	Diluent (PBS)	-1	0/10	---	---	0/10
		+1	0/10	---	---	0/10
		+2	0/10	---	---	0/10

[1]Groups of five mice per experiment were injected intraperitoneally with either 100, 50, or 25 ug of MAb in phosphate-buffered saline (PBS) 1 day before (-1), or 1 or 2 days after (+1, +2), challenge with 300 times the dose lethal for 50% of adult mice (10 plaque-forming units) of mouse-adapted Ebola Zaire virus.

[2]S/T, Number of mice that survived challenge/total number challenged.

Infect. Dis. 178, 651 (1998)). None of the tested MAbs were protective when 100 ug was administered 3 days after challenge (data not shown), when there are high viral titers and possibly irreversible damage of cells and organs.

Example 3

Competitive binding of Ebola GP MAbs

This study identified protective GP-specific MAbs that were classified into five groups on the basis of competitive binding assays. One protective MAb from each of these five different competition groups was chosen for further characterization and was submitted to ATCC as a representative of the competition group. Competitive binding between biotinylated and unlabeled MAbs for the GP of the Ebola Zaire 1995 virus was evaluated by ELISA. The results of the binding assays for the prototypical protective MAb from each competition group are depicted in Table 4. Except for one instance of one-way competition (between the group 4 and 5 MAbs), competition between labeled and unlabeled MAbs was reciprocal.

Table 4. Competitive Binding of Ebola GP MAbs

Biotinylated MAb	Competing Unlabeled MAb				
	13F6	6D8	12B5	13C6	6D3
13F6	0.3	0.8	0.5	1.0	1.2
6D8	0.6	0.2	0.6	0.6	0.7
12B5	0.3	0.3	0.1	0.2	0.3
13C6	0.6	0.7	0.6	0.1	0.5
6D3	0.5	0.6	0.6	0.1	0.2

Nonprotective MAbs were identified that bound competitively with protective MAbs in groups 1, 4 and 5 (Table 5). All of the antibodies that were completely protective were of the IgG2a subclass, whereas the competing nonprotective MAbs in groups 1 and 4 were of the IgG1 or IgG3 subclasses. Furthermore, the group 3 MAb (12B5), which was only

proteins and by indirect immunofluorescence assay (IFA) for their ability to react with BHK cells infected with EboGP-VRP. The initial screening by ELISA and IFA resulted in 616 positive cultures. Forty of these cultures were chosen for further analysis and were cloned twice by limited dilution. Twenty-seven of the hybridoma cultures continued to react specifically with the Ebola GP throughout the cloning process. The other hybridoma cultures were either lost in the cloning process or produced antibodies that reacted with cellular proteins. Isotype analysis of the MAbs produced by the 27 hybridoma cultures demonstrated that 6 were of the IgG1 subclass, 17 were of the IgG2a subclass, 2 were of the IgG2b subclass, 1 was of the IgG3 subclass, and 1 was an IgA antibody. All of the MAbs produced by these hybridoma cultures reacted with Ebola GP by IFA and 23 of the 27 MAbs reacted with Ebola virions by ELISA. These hybridoma cell lines were cultured in serum-free medium (Life Technologies, Grand Island, N.Y.) in Integra Celline flasks (Integra Biosciences, Inc., Ijamsville, MD). The IgG MAbs were purified from the supernatants on Protein G affinity columns (Amersham Pharmacia, Piscataway, N.J.), dialyzed in phosphate-buffered saline, and measured using the BCA protein assay (Pierce, Rockford, IL).

Example 2

Protective efficacy of Ebola GP MAbs in vivo

In order to determine the protective efficacy of the Ebola GP MAbs, purified MAbs were evaluated for their ability to protect mice from a lethal Ebola challenge (Table 3). In addition, competitive binding assays were performed to determine if the MAbs were recognizing the same epitope or unique epitopes on the

partially protective, was IgG1. Thus, antibody subclass may be an important factor in protection. Murine IgG2a binds complement more effectively than IgG1 or IgG3 and varies in its affinity for different Fc receptors (H. Waldmann, Ann. Rev. Immunol. 7, 407 (1989)). The subclass of the antibody may therefore affect the ability of the MAbs to resolve Ebola infections, for example by lysing infected cells through the classical complement pathway or by binding Fc receptors on cellular effectors of antibody-dependent-cell-mediated cytotoxicity.

Table 5. Competition Groups of Ebola GP MAbs

Competition Group	MAb Designation	MAb Isotype	Protection In Mice
1	13F6	IgG2a	Yes
1	6E3	IgG1	No
2	6D8	IgG2a	Yes
2	7E10	IgG2a	Yes
2	17E11	IgG2a	Yes
3	12B5	IgG1	Yes
4	13C6	IgG2a	Yes
4	11H12	IgG2a	Yes
4	9H6	IgG2a	Yes
4	1G8	IgG2a	Yes
4	12E12	IgG3	No
5	6D3	IgG2a	Yes
5	8C10	IgG2a	No
5	3H8	IgG2a	No

Alternatively, the affinity of an antibody for its epitope, possibly influenced by post-translational modifications such as glycosylation, may be an important determinant of protective efficacy. For instance, although group 5 consisted of three IgG2a MAbs, only 6D3 (Table 5) was protective. This MAb bound to Ebola virus at 10-fold lower concentrations than the two nonprotective MAbs (data not shown). In addition, the protective MAb in competition group 1

was more effective than the nonprotective MAb in competition assays (data not shown), suggesting that protective MAbs may have higher affinities for the epitope than nonprotective MAbs.

Example 4

Epitopes bound by Ebola GP MAbs

To further analyze the binding characteristics of the protective MAbs, MAbs were examined by radioimmunoprecipitation, western blot analysis, and peptide-binding assays. MAbs in competition groups 1, 2, and 3 immunoprecipitated GP, but not sGP, from supernatants of cell cultures infected with either Ebola Zaire virus or EboGP-VRPs (Fig. 2), and reacted only with GP1 in western blots (data not shown). The sequences bound by these MAbs were identified by means of synthetic peptides immobilized on membranes and were confirmed with soluble peptides in competition ELISAs (Table 2). These protective MAbs bound linear epitopes within a region of 106 amino acids in the C-terminal portion of GP1. This region is poorly conserved among Ebola viruses and is not shared with sGP. The epitopes bound by MAbs in competition groups 1 and 2 are separated by only three amino acids (Table 2).

In contrast, MAbs in competition groups 4 and 5 immunoprecipitated both GP and sGP from supernatants of infected cells (Fig. 2) but did not bind GP on western blots under reducing conditions or react with any of the synthetic Ebola GP peptides immobilized on membranes (data not shown). These epitopes are therefore discontinuous or require a specific conformation for binding, and are located within the N-terminal 295 amino acids that are identical between sGP and GP1.

Example 5

Cross-reactivity of MAbs with Ebola virus subtypes

All of the MAbs in this report were generated against the Zaire strain of the Ebola virus. To determine if the MAbs cross-react with the GP of other Ebola strains that are human pathogens, the reactivities of the MAbs with the Zaire 1976, Zaire 1995, Ivory Coast, and Sudan isolates of Ebola virus were compared by ELISA. The Reston strain of the Ebola virus has not been demonstrated to be a human pathogen and was therefore not tested in this report.

When the MAbs were tested for reactivity with the Ebola viruses that are human pathogens, MAbs in competition groups 1, 2, and 3 bound to the two Zaire isolates that have caused the most devastating outbreaks, but did not bind to the Ivory Coast or Sudan viruses (Table 2). All of the MAbs in competition groups 4 and 5 bound to the Ebola Zaire and Ivory Coast viruses. Furthermore, MAbs in competition group 4, but not group 5, also bound to Ebola Sudan (Table 2). These results suggest that it is possible to elicit by vaccination, or produce for therapeutic use, antibodies protective against all Ebola viruses that are pathogenic for humans.

Example 6

In vitro neutralization of Ebola virus by Ebola GP MAbs

In order to determine if the protective MAbs were able to neutralize Ebola virus in vitro, purified MAbs were evaluated for their ability to inhibit plaque formation by Ebola virus. None of the protective MAbs inhibited plaque formation in the absence of complement (Table 6). In the presence of complement,

only MAbs in competition groups 2 and 4 neutralized the virus (80% at 6.25 ug/ml, Table 6). MAb 12B5 (competition group 3) did not reduce the number of plaques, but did reduce plaque size (Table 6), suggesting that it restricted subsequent infection of adjacent cells. These results demonstrated that the ability of the MAbs to inhibit plaque formation by Ebola virus, a standard assay of virus neutralization, did not always predict their protective efficacy.

Table 6. In Vitro Neutralization Activity of Ebola GP MAbs.

MAb Designation	Competition Group	Neutralization w/Complement	Neutralization w/o Complement
13F6	1	None	None
6D8	2	6.25 ug/ml	None
12B5	3	None*	None*
13C6	4	6.25 ug/ml	None
6D3	5	None	None

*Plaque size was reduced (pinpoint plaques) compared with control plaques. Plaque assays were performed using confluent Vero-E6 cells. To evaluate the presence of Ebola-neutralizing antibodies, four-fold serial dilutions of MAbs (starting at 100 µg/ml) were mixed with 100 pfu of mouse-adapted Ebola virus at 37°C for 1 h, and used to infect Vero E6 cells. Cells were covered with an agarose overlay (Moe, J. et al. (1981) J. Clin. Microbiol. 13:791-793) and a second overlay containing 5% neutral red solution in PBS or agarose was added 6 days later. Plaques were counted the following day. In some experiments, guinea pig complement (5% final concentration) was added to facilitate antibody-complement lysis of infected cells. Endpoint titers were determined to be the last dilution of MAb that reduced the number of plaques by 80% compared with the control wells.

Example 7

Combinations of MAbs can reduce the effective dose required for in vivo protection from Ebola virus

Table 3 demonstrated that MAbs recognizing single epitopes on the Ebola GP were capable of protecting mice from lethal Ebola challenge when 100 ug of Mab was administered either 1 day before or up to 2 days after receiving a lethal dose of Ebola virus. To

determine if combinations of MAbs from different competition groups could reduce the effective dose required for protection, MAbs from 3 to 5 different competition groups were mixed and evaluated for their ability to protect BALB/c mice from a lethal challenge with mouse-adapted Ebola Zaire virus. The concentrations of MAbs chosen for these studies were below the concentrations at which the individual MAbs were able to protect all of the mice from death.

Administration of 37.5 ug of a combination of 3 different MAbs (12.5 ug of MAb 13F6, 12.5 ug of MAb 6D8, and 12.5 ug of MAb 13C6) one day prior to Ebola challenge was able to protect 4/5 mice from lethal disease (Table 7A). When the same 3 MAbs were administered at concentrations of either 25 ug or 50 ug of each MAb/mouse (for a total of 75 ug or 150 ug of MAb/mouse, respectively), 100% of the mice were protected from lethal challenge. Therefore, prophylactic administration of a combination of MAbs that recognize different epitopes on the Ebola GP is more efficient than single MAbs at protecting against lethal challenge.

To determine if combinations of MAbs were also effective therapeutically, groups of 5 BALB/c mice were injected with various combinations of MAbs 2 days after a lethal challenge with mouse-adapted Ebola Zaire virus. Combinations of MAbs 13F6, 6D8, and 13C6 were able to protect 4/5 mice when administered at a concentration of 25 ug or 50 ug of each MAb/mouse (Table 7B). When combinations of MAbs 13F6, 6D8, 12B5, 13C6, and 6D3, representing the 5 different competition groups for protective Ebola GP MAbs, were administered at a concentration of either 12.5 ug or 25 ug of each MAb/mouse 2 days after Ebola challenge, all of the mice survived (Table 7B). Therefore,

combinations of MAbs which recognize different epitopes on the Ebola GP are effective both prophylactically when administered one day prior to Ebola challenge and therapeutically when administered 2 days after Ebola challenge when significant viral replication has already occurred in the host.

Table 7. Protective Efficacy of Combinations of Ebola GP Monoclonal Antibodies

A. Prophylactic Administration (1 Day Prior to Challenge):

MAbs Administered	MAb Dose[1] (ug)	Survivors/ Total	MDD[2]
13F6, 6D8, and 13C6	12.5 of each (37.5 total)	4/5	9*
13F6, 6D8, and 13C6	25 of each (75 total)	5/5	---
13F6, 6D8, and 13C6	50 of each (150 total)	5/5	---
None (PBS)	---	0/5	6.6±2.0

B. Therapeutic Administration (Day 2 Post-Challenge):

MAbs Administered	MAb Dose[1] (ug)	Survivors/ Total	MDD[2]
13F6, 6D8, and 13C6	25 of each (75 total)	4/5	6*
13F6, 6D8, and 13C6	50 of each (150 total)	4/5	7*
13F6, 6D8, 12B5, 13C6 and 6D3	12.5 of each (62.5 total)	5/5	---
13F6, 6D8, 12B5, 13C6 and 6D3	25 of each (125 total)	5/5	---

[1]MAbs were administered intraperitoneally into BALB/c mice either 1 day before or 2 days after challenge with mouse-adapted Ebola Zaire virus.
[2]MDD, Mean Day of Death
*n=1

This report thus demonstrates that antibodies are a feasible option for the design of safe and standardized treatments for Ebola infections. However, antibody specificity and the ability to neutralize the Ebola virus in vitro cannot be used as sole predictors of protective efficacy. Protection may depend on the proper specificity, isotype, and/or affinity of the antibody.

What is claimed is:

1. An antibody which recognizes Ebola virus GP.

2. The antibody according to claim 1, wherein said antibody is a monoclonal antibody.

3. The antibody according to claim 1, wherein the antibody inhibits Ebola virus infection in a subject in vivo.

4. The antibody according to claim 1, wherein the antibody ameliorates symptoms of Ebola virus infection when said antibody is administered to a subject after infection with the Ebola virus.

5. The antibody according to claim 1, wherein the antibody binds Ebola virus in vitro.

6. The antibody according to claim 1, wherein the antibody immunoprecipitates GP from supernatants or cell lysates of cell cultures infected with Ebola virus.

7. The antibody according to claim 1, wherein the epitope of said antibody is within SEQ ID NO: 3.

8. The antibody according to claim 7 wherein said epitope is SEQ ID NO:4.

9. The antibody according to claim 8 wherein said epitope is defined as SEQ ID NO:5.

10. The antibody according to claim 7 wherein said epitope is SEQ ID NO:6.

11. The antibody according to claim 10 wherein said epitope is defined as SEQ ID NO:7.

12. The antibody according to claim 7 wherein said epitope is SEQ ID NO:8.

13. The antibody according to claim 12 wherein said epitope is defined as SEQ ID NO:9.

14. The antibody according to claim 2, wherein said antibody is produced by hybridoma cell line EGP 13F6-1-2 with Accession no. PTA-373.

15. An antibody which competes with the antibody of claim 14 for binding to Ebola virus GP.

16. The antibody according to claim 2, wherein said antibody is produced by hybridoma cell line EGP 6D3-1-1 Accession no. PTA-374.

17. An antibody which competes with the antibody of claim 16 for binding to Ebola virus GP.

18. The antibody according to claim 2, wherein said antibody is produced by hybridoma cell line EGP 13C6-1-1 Accession no. PTA 375.

19. An antibody which competes with the antibody of claim 18 for binding to Ebola virus GP.

20. The antibody according to claim 2, wherein said antibody is produced by hybridoma cell line EGP 6D8-1-2 Accession no. PTA 376.

21. An antibody which competes with the antibody of claim 20 for binding to Ebola virus GP.

22. The antibody according to claim 2, wherein said antibody is produced by hybridoma cell line EGP 12B5-1-1 Accession no. PTA 436.

23. An antibody which competes with the antibody of claim 22 for binding to Ebola virus GP.

24. A mixture comprising Ebola virus antibodies comprising one or more antibodies selected from the group consisting of

an antibody produced by hybridoma EGP 13F6-1-2 accession no. PTA 373;

an antibody produced by hybridoma EGP 6D3-1-1 accession no. PTA 374;

an antibody produced by hybridoma EGP 13C6-1-1 accession no. PTA 375;

an antibody produced by hybridoma EGP 6D8-1-2 accession no. PTA 376; and

an antibody produced by hybridoma EGP 12B5-1-1 accession no. PTA 436.

25. A mixture according to claim 24 wherein said mixture prevents Ebola virus infection in a subject upon administration to said subject.

26. A therapeutic composition for ameliorating symptoms of Ebola virus infection comprising the mixture of claim 24, and a pharmaceutically acceptable excipient.

33. The method according to claim 31 wherein said monoclonal antibodies compete for binding to GP with an antibody chosen from the group consisting of MAb 13F6, MAb 6D3, MAb 13C6, MAb 6D8, and MAb 12B5.

34. A method for detecting Ebola virus according to claim 32, wherein said sample is a biological sample.

35. A method of treating Ebola virus infection comprising administering to a patient in need of said treatment an effective amount of a composition comprising one or more monoclonal antibodies selected from the group consisting of: MAb 13F6, MAb 6D3, MAb 13C6, MAb 6D8, and MAb 12B5.

36. The method according to claim 35 wherein said composition comprises antibodies which compete for binding to GP with an antibody chosen from the group consisting of MAb 13F6, MAb 6D3, MAb 13C6, MAb 6D8, and MAb 12B5.

37. A kit for detecting Ebola virus in a biological sample, said kit comprising:

(1) a container holding at least one monoclonal antibody selected from the group consisting of MAb13F6, MAb 6D3, MAb 13C6, MAb 6D8, and MAb 12B5, and

(2) instructions for using said antibody for the purpose of binding to Ebola virus to form an immunological complex and detecting the formation of the immunological complex such that presence or absence of immunological complex correlates with presence or absence of Ebola virus in said sample.

38. The kit according to claim 37 comprising antibodies which compete for binding to Ebola GP with an antibody chosen from the group consisting of MAb 13F6, MAb 6D3, MAb 13C6, MAb 6D8, and MAb 12B5.

39. A vaccine for Ebola virus comprising one or more antigenic peptide epitopes recognized by any monoclonal antibody selected from the group consisting of MAb13F6, MAb6D3, MAb13C6, MAb6D8, and MAb12B5.

40. The vaccine according to claim 39 wherein said peptides are chosen from the group consisting of, SEQ ID NO: 3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 and SEQ ID NO:9.

41. A vaccine according to claim 40 wherein said peptides comprise the region of Ebola GP encompassing amino acid residues 389-493 (SEQ ID NO:3).

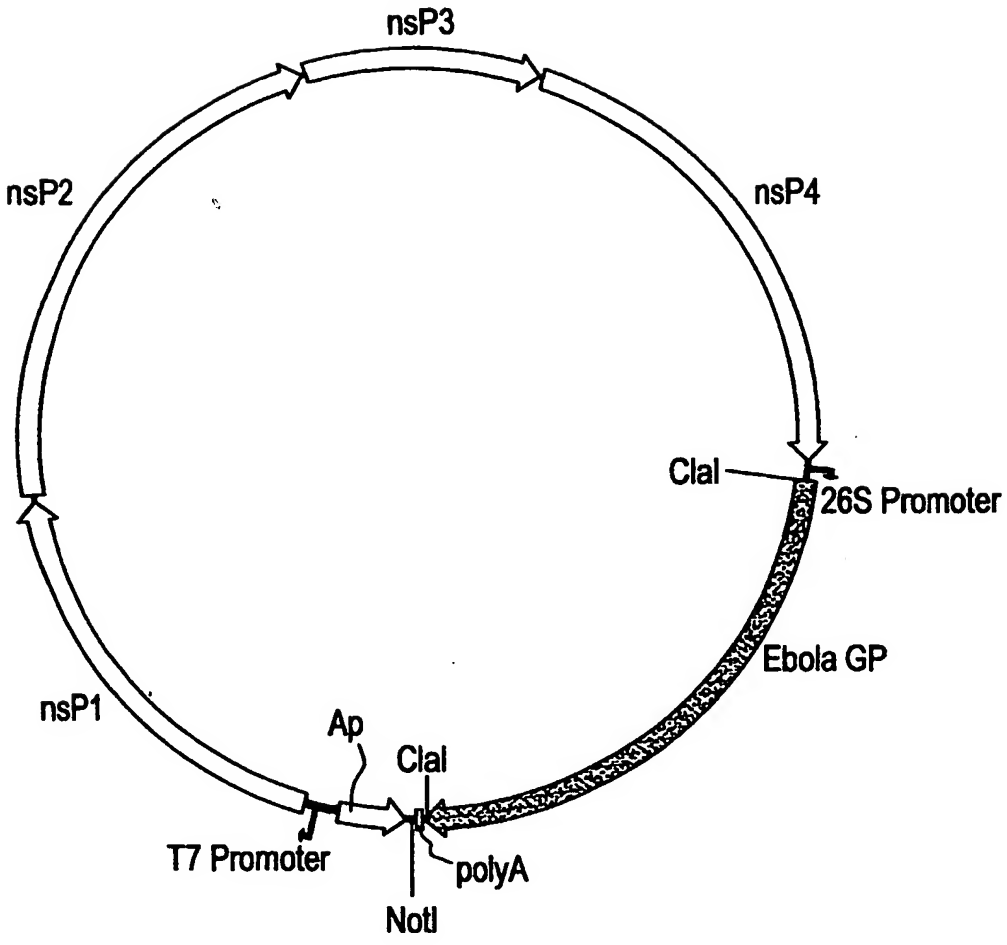
42. A pharmaceutical composition comprising a peptide encoded by any of SEQ ID NO:3-10, in a pharmaceutically acceptable amount, in a pharmaceutically acceptable carrier and/or adjuvant.

43. An antiidiotypic antibody produced from any of the monoclonal antibodies selected from the group consisting of MAb13F6, MAb 6D3, MAb 13C6, MAb 6D8, and MAb 12B5.

44. An antiidiotypic antibody produced from an antibody which competes for binding to GP with an antibody selected from the group consisting of MAb13F6, MAb 6D3, MAb 13C6, MAb 6D8, and MAb 12B5.

45. An Ebola virus vaccine comprising one or more antiidiotypic antibodies according to claim 39, and an acceptable carrier.

FIG. 1


nsP3
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nsP4
ClaI
26S Promoter
Ebola GP
nsP1
Ap
ClaI
T7 Promoter
polyA
NotI

FIG. 2B

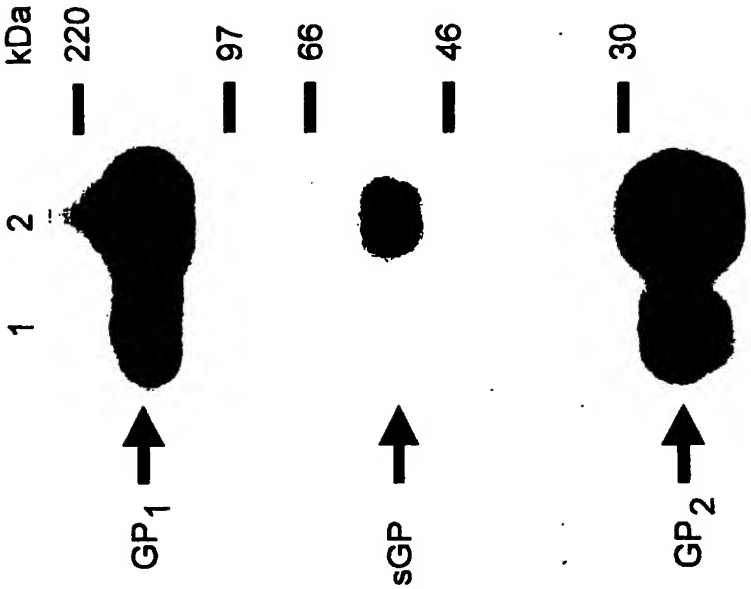
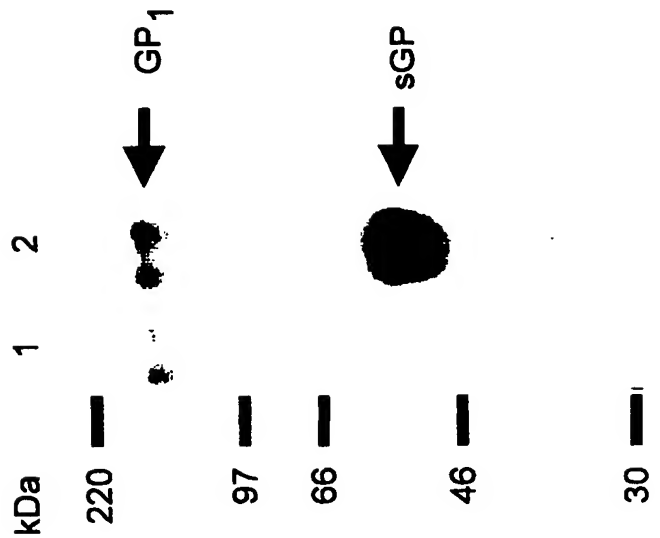


FIG. 2A



SEQUENCE LISTING

Sequence ID NO 1: (Ebola GP DNA sequence in VEE replicon):

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TTGGGTAATT ATCCTTTTCC
AAAGAACATT TTCCATCCCA CTTGGAGTCA TCCACAATAG CACATTACAG
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GGACTACTCA GGATGAAGGT GCTGCAATCG GACTGGCCTG GATACCATAT
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ACCACATGAT TGGACCAAGA ACATAACAGA CAAAATTGAT CAGATTATTC
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TTACAGGCGT TGTAATTGCA GTTATCGCTT TATTCTGTAT ATGCAAATTT
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Sequence ID NO 2: (Ebola GP amino acid sequence from VEE replicon):

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Arg Thr Ser Phe
Phe Leu Trp Val Ile Ile Leu Phe Gln Arg Thr Phe Ser Ile Pro
Leu Gly Val Ile
His Asn Ser Thr Leu Gln Val Ser Asp Val Asp Lys Leu Val Cys
Arg Asp Lys Leu
Ser Ser Thr Asn Gln Leu Arg Ser Val Gly Leu Asn Leu Glu Gly
Asn Gly Val Ala
Thr Asp Val Pro Ser Ala Thr Lys Arg Trp Gly Phe Arg Ser Gly
Val Pro Pro Lys
Val Val Asn Tyr Glu Ala Gly Glu Trp Ala Glu Asn Cys Tyr Asn
Leu Glu Ile Lys
Lys Pro Asp Gly Ser Glu Cys Leu Pro Ala Ala Pro Asp Gly Ile
Arg Gly Phe Pro
Arg Cys Arg Tyr Val His Lys Val Ser Gly Thr Gly Pro Cys Ala
Gly Asp Phe Ala
Phe His Lys Glu Gly Ala Phe Phe Leu Tyr Asp Arg Leu Ala Ser
Thr Val Ile Tyr
Arg Gly Thr Thr Phe Ala Glu Gly Val Val Ala Phe Leu Ile Leu
Pro Gln Ala Lys
Lys Asp Phe Phe Ser Ser His Pro Leu Arg Glu Pro Val Asn Ala
Thr Glu Asp Pro
Ser Ser Gly Tyr Tyr Ser Thr Thr Ile Arg Tyr Gln Ala Thr Gly
Phe Gly Thr Asn
Glu Thr Glu Tyr Leu Phe Glu Val Asp Asn Leu Thr Tyr Val Gln
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Phe Thr Pro Gln Phe Leu Leu Gln Leu Asn Glu Thr Ile Tyr Thr
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Ser Asn Thr Thr Gly Lys Leu Ile Trp Lys Val Asn Pro Glu Ile
Asp Thr Thr Ile
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Ile Arg Ser Glu
Glu Leu Ser Phe Thr Val Val Ser Asn Gly Ala Lys Asn Ile Ser
Gly Gln Ser Pro

Ala Arg Thr Ser Ser Asp Pro Gly Thr Asn Thr Thr Thr Glu Asp
His Lys Ile Met
Ala Ser Glu Asn Ser Ser Ala Met Val Gln Val His Ser Gln Gly
Arg Glu Ala Ala
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Leu Ala Trp Ile
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SEQ ID NO:9
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Sequence ID NO 10:
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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 00/23790

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/10 A61K39/42 C12N5/20 G01N33/569 G01N33/577
A61K39/12 C07K16/42 A61P31/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EMBASE, CHEM ABS Data, WPI Data, PAJ, EPO-Internal, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	T. MARUYAMA ET AL.: "Recombinant human monoclonal antibodies to Ebola virus." JOURNAL OF INFECTIOUS DISEASES, vol. 179, no. suppl. 1, February 1999 (1999-02), pages S235-S239, XP000971336 Chicago, IL, USA page S235, right-hand column, line 25 -page S236, left-hand column, line 4 results and discussion -/--	1-6,30, 31

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 December 2000	11/01/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nooij, F

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 00/23790

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	A. SANCHEZ ET AL.: "Biochemical analysis of the secreted and virion glycoproteins of Ebola virus." JOURNAL OF VIROLOGY, vol. 72, no. 8, August 1998 (1998-08), pages 6442-6447, XP002156122 Washington, DC, USA abstract page 6444, left-hand column, line 5 -right-hand column, line 23 figures 2,7	1,5-7
A	L. XU ET AL.: "Immunization for Ebola virus infection." NATURE MEDICINE, vol. 4, no. 1, January 1998 (1998-01), pages 37-42, XP002131515 New York, NY, USA abstract	39-42
A	V. VOLCHKOV ET AL.: "The envelope glycoprotein of Ebola viris contains an immunosuppressive-like domain similar to oncogenic retroviruses." FEBS LETTERS, vol. 305, no. 3, July 1992 (1992-07), pages 181-184, XP002156123 Amsterdam, The Netherlands figures 1,2	1-45
P,X	J. WILSON ET AL.: "Epitopes involved in antibody-mediated protection from Ebola virus." SCIENCE, vol. 287, no. 5458, 2000, pages 1664-1666, XP002156124 Washington, DC, USA the whole document	1-38
P,X	WO 00 00617 A (U.S. MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES) 6 January 2000 (2000-01-06) examples claims	39-42

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-38

   Antibodies which recognize Ebola virus GP, and their diagnostic and therapeutic use.

2. Claims: 39-42

   Vaccine for Ebola virus comprising epitopes from Ebola GP, and a pharmaceutical composition comprising said vaccine

3. Claims: 43-45

   Antiidiotypic antibodies produced from mAbs against Ebola virus GP, and a vaccine comprising one or more of said antiidiotypic antibodies.

INTERNATIONAL SEARCH REPORT

ormation on patent family members

Inter nal Application No
PCT/US 00/23790

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0000617 A	06-01-2000	AU 4709099 A	17-01-2000
		AU 5084499 A	17-01-2000
		WO 0000616 A	06-01-2000